RESEARCH ARTICLE

MANAGEMENT OF SEED-BORNE FUNGI OF FRENCH BEAN (PHASEOLUS VULGARIS L.) WITH FUNGICIDAL TREATMENT

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Abstract: Seeds of French bean (*Phaseolus vulgaris* L.) are infected during storage conditions, which affect the germination percentage. Seeds were evaluated using the agar plate method to determine the fungal association. Seven fungal species were isolated from the internal and external seed surfaces of French bean, viz., *Aspergillus flavus*, *Aspergillus niger*, *Fusarium solani*, *Mucor* spp., *Penicillium* spp., *Rhizopus oryzae*, and *Rhizopus stolonifer*. Seeds were treated with four fungicides viz. Captan, Dithane M45, Zim50, and Saff before germination to study the efficacy against seed-borne fungi. Out of four fungicides used, Saff and Dithane M-45 were found effective to control seed mycoflora of French Bean. Treated seeds showed better germination percentage as well as root and shoot length than control.

Keywords: Fungicide, Management, Mycoflora, *Phaseolus vulgaris* L., Seed-borne

INTRODUCTION

French bean (*Phaseolus vulgaris* L.) belongs to the family leguminaceae and it is also known as common bean, kidney bean, dwarf bean, navy bean, snap bean, string bean, garden bean, and edible bean in different part of India. French beans are grown winter crop in plains, while it can be grown all through the year except winter, in hills. It cannot withstand drought as well as very near rainfall and frost. It is the most commonly grown bean and can be consumed as a vegetable when pods are immature or as dry pulse after maturity. Because of its nutritive value, it now is the most popular bean vegetable and pulse crop in India. The dried seeds are highly nutritious, containing 25% protein, 50% starch, 2.0% fat, and 3.0% minerals (Chatfield, 1949). French bean was also cultivated for green managing and erosion control throughout the world.

Seeds play a vital role in the healthy production of crops, which are carriers of some important seed-borne diseases which cause considerable losses in yield. Seed-borne fungi are chiefly responsible for the deterioration of seed in storage and thus they remarkably reduce the germination potential of stored seeds (Shah and Jain, 1993). After harvesting seeds are stored at different storage conditions and if these storage conditions are not proper, various microorganisms like bacteria, viruses, nematodes, and fungi interact with these seeds. Among these microorganisms, fungi play a dominant role in decreasing the quality and longevity of the seeds. Several seed-borne fungi, including species of *Alternaria*, *Aspergillus*, *Fusarium*, and *Peniciliium* have been detected as seedling pathogens of cereals (Fahim, Barakat, and Aly, 1983). Many fungi are serious parasites of seed primordia, maturing and stored seeds and grains and their invasion can result in various abnormalities including, reduce yields of seed in both quantitatively and qualitatively, discolorations, decrease germinability, mycotoxin production, and total decay (Castillo *et. al*., 2004). The fungal pathogens play a major role in the development of diseases or many important field and horticulture crops; resulting in severe plant yield losses.

Studies on seed mycoflora have greatly increased in recent times because of their importance in toxin production, seed deteriorating agents, and disease carriers (Tamuli and Nath, 2007). Seed mycoflora also affects germination (Tamuli and Baruah, 2001). Since this valuable crop is propagated by seed, an investigation was carried out to study the seed mycoflora and its control.

MATERIALS AND METHOD

Collection of seeds:

Seeds of French bean were collected from different local farmers of Tezpur. The seeds were collected in a sterile polythene bag and the collected seeds were mixed to form a composite sample. The seeds were preserved at room temperature during the studies.

Isolation and identification of mycoflora:

The surface mycoflora of selected seeds were isolated by the agar plate method as recommended by the International Seed Testing Association ISTA.

Agar Plate Method:

In Northern Ireland, Musket first used this method for seed health management 28. In this method, pre-sterilized petriplates were poured with 15 ml of autoclaved Potato Dextrose Agar (PDA). On cooling the medium, ten seeds per plate of the sample to be studied were equidistantly placed aseptically. Then plates were incubated at 28±1ºC with 12 hours alternating cycles of light and darkness. After that plates were examined after 8 days of incubation. The fungi occurring on seeds plated on agar plate were preliminarily identified based on sporulation characters. Detail examination of fungal characters

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was done by using a compound microscope and identification was also confirmed. The total percentage of fungal incidence of fungi was calculated by using the following formula-

$$\text{Frequnecy} = \frac{\text{Total no of seeds in which particular fungus appeared}}{\text{Total no. of seeds studied}} \times 100$$

Detection of seed mycoflora:

Pre-sterilized petriplates were poured equally with autoclaved Potato Dextrose Agar (PDA) under a laminar airflow chamber. On cooling the medium, six seeds per plate of the sample to be studied were equidistantly placed aseptically in petridishes. All six seeds per petridish and the petridishes were incubated at 25 ± 1^0C. On the fourth day of incubation, observations were made for determining the various fungal growth. The identification and further confirmation of seed-borne fungi were made by preparing slides of the fungi.

Application of fungicides in controlling the seed mycoflora:

Four fungicides viz. Captan, Dithane M45, Zim50, and Saff were selected for this study and 0.3 percent (prepared in sterilized water) were made. Seeds were treated for 30 minutes. Pre-sterilized petridishes were poured equally with autoclaved PDA under a laminar airflow chamber. On cooling the medium, five seeds per plate of the fungicide treated seeds were equidistantly placed aseptically in four petridishes. And the remaining one petridish were kept under control. All petridishes were incubated at 25 ± 1^0C. After incubation, observations were made.

Study of germination percentage and root/shoot length:

To study the seed germination, four fungicides viz. Captan, Dithane M45, Zim50, and Saff were selected and 0.3 percent (prepared in sterilized water) of each were used. 120 seeds were treated with sterilized distilled water served as a control group. Seed germination was studied by plating the treated and non-treated control seeds on sterilized moist blotting papers. The rate of germination was recorded after 6 days of plating. The germination percentage was recorded following ISTA rules (Naseri and Mousavi, 2008).

$$\text{Germination (\%)} = \frac{\text{No.of seed germinated}}{\text{No.of seeds tested}} \times 100$$

After, the seed germinations, the length of roots and shoots were recorded for several days.

RESULTS AND DISCUSSION

The seeds of French Bean harbored all together seven fungal species, viz.; *Aspergillus flavus*, *Aspergillus niger*, *Fusarium solani*, *Mucor* spp., *Penicillium* spp., *Rhizopus oryzae*, *Rhizopus stolonifer*. The efficacy of the fungicides showed that at 0.3% concentration of Dithane M45 and Saff almost eliminated the seed mycoflora of French bean, which is followed by Zim50 and Captan. The untreated (control) seeds showed more percentage of infested seeds.

Table 1. Efficacy of different fungicides in controlling the seed mycoflora of French bean.

Fungicides (0.3%)	Percentage of seed showing incidence after treatment with fungicides.						
	Af	An	Fs	Ms	Ps	Ro	Rs
Captan	7	3	5	15	6	10	9
Zim 50	2	–	3	7	8	–	8
DithaneM45	–	–	–	–	–	–	3
Saff	–	–	–	1	–	–	4
Control	22	27	35	38	19	31	40

Af =*Aspergillus flavus,* An=*Aspergillus niger,* Fs=*Fusarium solani*, Ms=*Mucor* spp., Ps=*Penicillium* spp., Ro=*Rhizopus oryzae,* Rs= *Rhizopus stolonifer.*

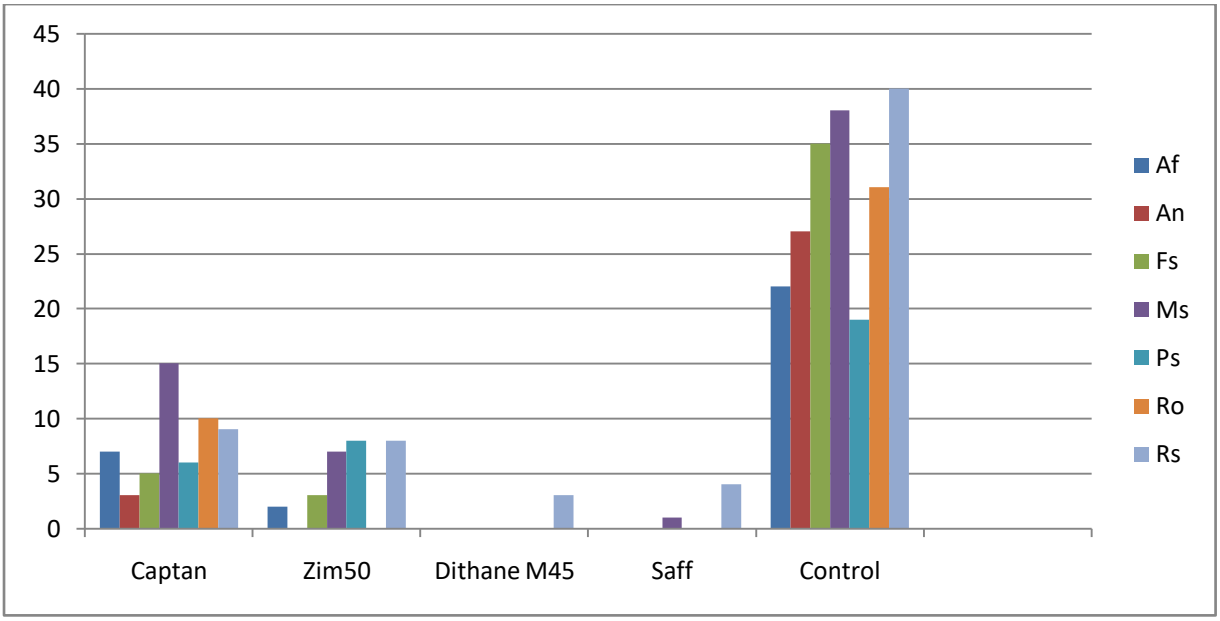


Figure 1: Efficacy of different fungicides in controlling the seed mycoflora of French bean.

Seed-borne fungi affect the germinability of seed (Feliciano *et.al*, 1981). Seed mycoflora directly injures the seed resulting in a reducing percentage of germination. The untreated seeds of French bean showed 70% of germination percentage, whereas Captan showed 93.33%. Similarly, Zim 50, Dithane M45, and Saff showed 93.33%, 96.67%, and 93% germination percentage respectively. Amongst all the fungicides, Dithane M45 proved to be the best one for improving seed germinability. The improved germination of seed after fungicidal treatments, on the whole, is due to the elimination of fungi because they secrete mycotoxins which are responsible for the reduction of seed germination.

The average root and shoot length of untreated seed was found 4.2 c.m and 3.2 c.m respectively, whereas the average root and shoot length of the treated seeds were found 5.0 c.m and 4.3 c.m (Captan), 5.2 c.m and 4.0 c.m (Zim 50), 5.7 c.m and 4.8 c.m (Dithane M45) and 5.5 c.m and 4.6 c.m (Saff).

After fungicidal treatment, the growth of root and shoot also increased as a comparison to control i.e. untreated seeds. It is only because of some fungicides also acted as growth-regulating hormones. Best results were found from Dithane M45 treated seeds.

Table 2. Effect of fungicides on seed germination percentage and root/shoot length of French bean.

Treatment (0.3%)	Germination (%)	Root length (Avg.) (c.m)	Shoot length (Avg.) (c.m)
Captan	93.33	5.0	4.3
Zim 50	93.33	5.2	4.0
Dithane M45	96.67	5.7	4.8
Saff	93	5.5	4.6
Control	70	4.2	3.2

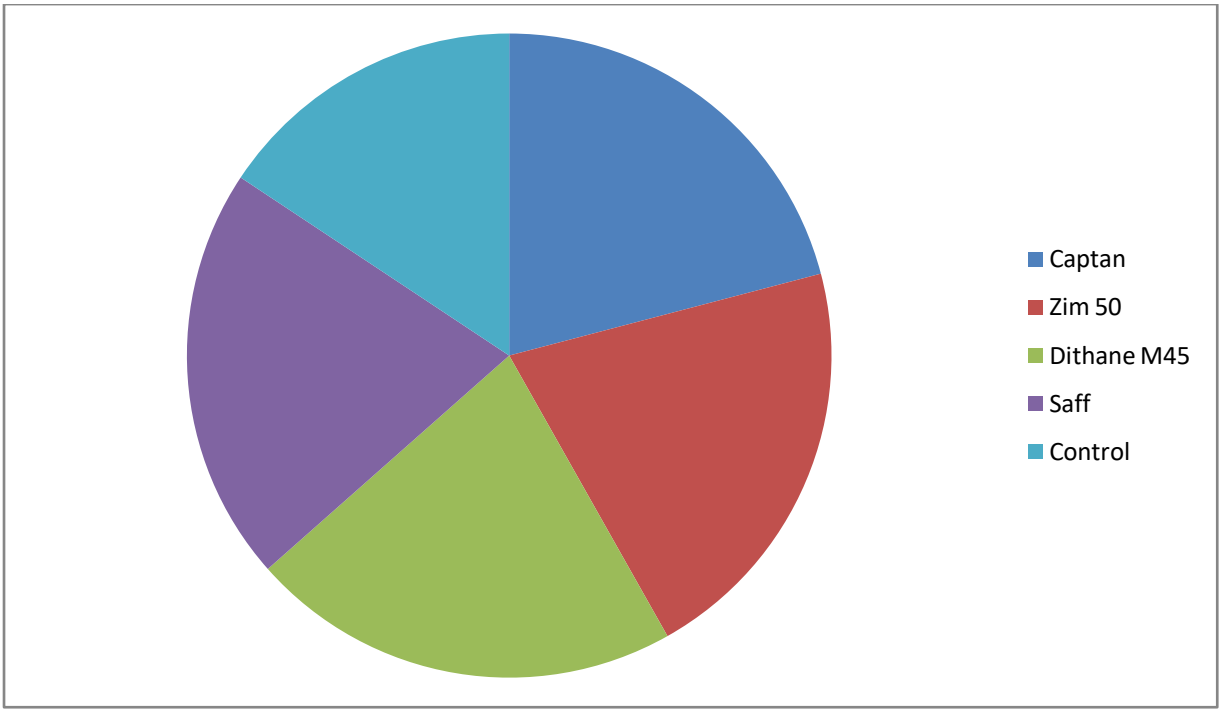


Figure 2: Effect of fungicides on seed germination.

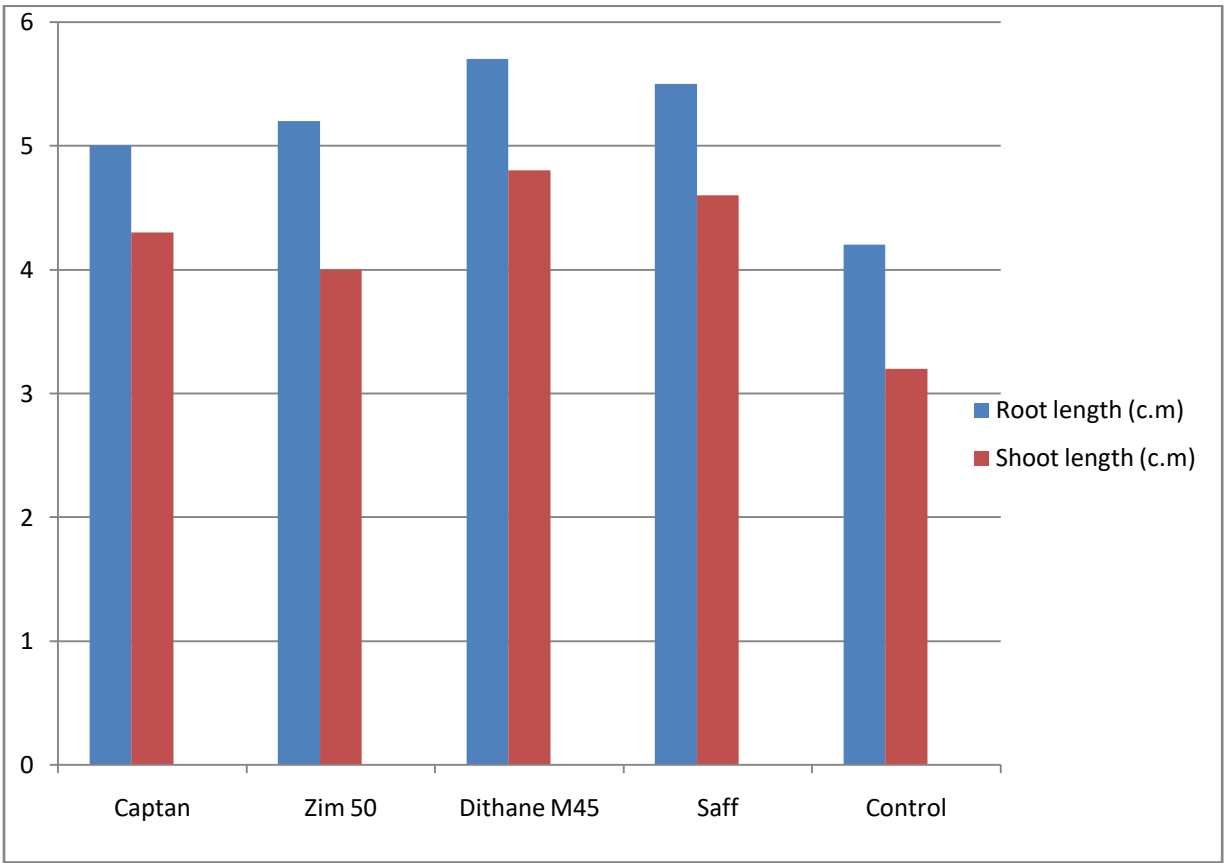


Figure 3: Effect of fungicides on root/shoot length.

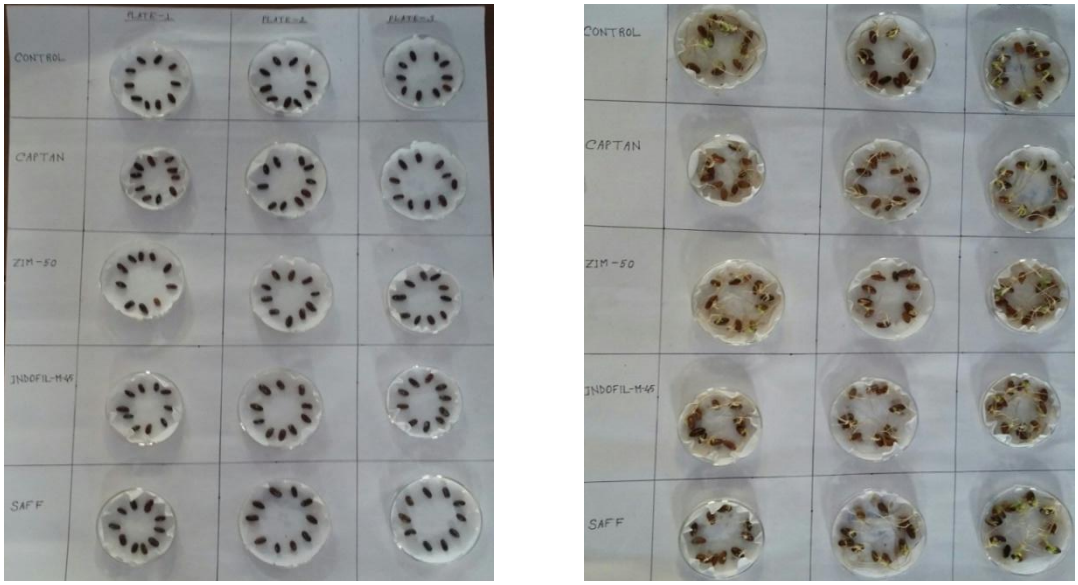
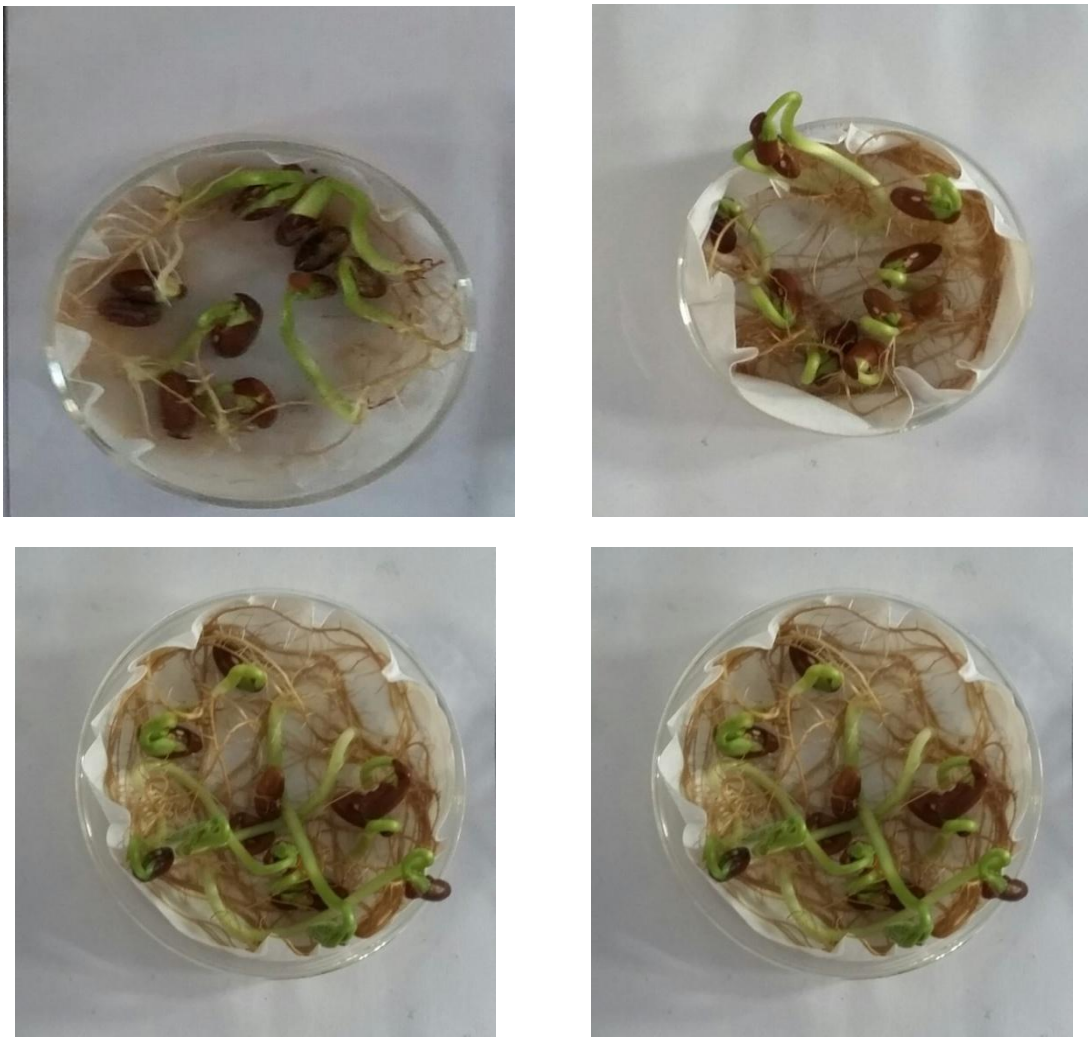


Figure 4: Germination study.



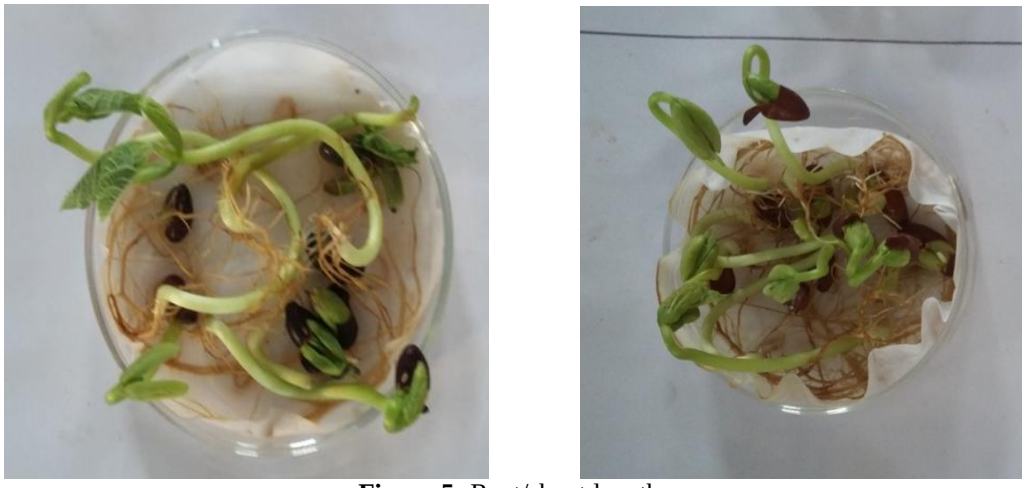


Figure 5: Root/shoot length

Results in this study showed that the germination percentage and root/shoot length of all the treated seed with fungicides was higher than that of the untreated ones. The improved germination of seed after fungicidal treatments, on the whole, is due to the elimination of fungi because they secrete mycotoxins which are responsible for the reduction of seed germination.

In this study, *Aspergillus flavus, Aspergillus niger, Fusarium solani, Mucor* spp., *Penicillium* spp., *Rhizopus oryzae*, and *Rhizopus stolonifer* were isolated from the French bean samples. A. Sinha, S.K. Singh and J. Quisar (1999) also reported *Aspergillus flavus, Aspergillus niger, Fusarium* spp, and *Penicillium* spp on French bean (Marcenaro D. and Valkonen J.P.T., 2016). The fungal species found in the present study were almost the same as reported earlier from Agarwood seeds (Shaheb M.R., *et. al.*, 2015). The similar fungal species were *Aspergillus flavus, Aspergillus niger, Fusarium solani, Penicillium* spp. and *Rhizopus stolonifer* Rani. P. *et.al* (1995) studied seed mycoflora of five spices in which species of *Aspergillus, Mucor* and *Rhizopus* spp. were most common. Again, *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp. were found by J.N. Srivastava *et.*al on Rajmah.

Although all the four fungicides tested were found to be quite effective in controlling the seed mycoflora of French bean, the best results were obtained from Dithane M45 seed treatment as it eliminated almost all the fungi and improved the seed germination potential of French bean. With the application of Dithane M45, the percentage of seed germination was enhanced up to 96.67% as compared to 70% in control. Deena *et.al* (1984) reported that Quintozene (0.15) and Mancozeb (0.15) as seed dressing could effectively control seed-borne mycoflora of chili (*Capsicum annum*). Singh and Singh (1986) suggested that *Vicia faba* seeds be treated with Dithane M45 for controlling the mycoflora associated with its seeds. Again, Tamuli and Boruah (2001) reported that Dithane M45 eliminated almost all the fungi and improved the seed germination potential of Agarwood. Two chemical fungicides were tested against some seed-borne fungi by Chauhan and Singh (2012) for the evaluation of inhibition of mycelial growth. Of the three fungicides used, Ridomil gave good results at all the concentrations against all the test fungi compared with Bavistin.Tejasvi and Kumar (2011) found that fly ash aqueous extract with increasing concentration (25-75%) stimulated the seed germination and seedling growth as compared to control. Therefore, our observations were found to be similar to the findings reported earlier by these workers in the case of mycoflora of other seeds. It is, therefore, suggested that French bean seed should be treated with Dithane M45 before sowing.

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